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(54) Title: VACCINE AND METHOD FOR TREATMENT OF CHLAMYDIAL INFECTIONS

(57) Abstract

A vaccine for treatment of chlamydial infections made up of a major outer membrane protein (MOMP) preparation and a lipopolysaccharide (LPS) preparation from a Chlamydia organism is provided. Methods of treating and immunizing animals against chlamydial infections are also provided.

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Vaccine and Method for Treatment of Chlamydial Infections

Background of Invention

Chlamydia trachomatis is the most prevalently sexually transmitted bacterial pathogen in the United States today. The complications resulting from chlamydial infections can be quite serious. Those infected may suffer from pelvic inflammatory disease, urethritis, urethral syndrome and urinary tract infections. It has also been confirmed that infection may result in spontaneous abortion in pregnant woman. In addition, chlamydial conjunctivitis and chlamydial pneumonia may occur in infants infected from their mothers as they pass through the birth canal.

Chlamydial infections in animals and humans are quite similar. The organism C. psittaci in animals primarily affects the mucosal epithelial cells of the eye and genital tract. After infection, a chronic carrier state typically develops with symptoms reoccurring during stress. Asymptomatic female animals carrying C. psittaci in epithelial cells of the distal genital tract have been shown to infect their newborn during parturition.Chlamydial infection in sheep is an economically devastating disease in many countries. Ovine chlamydial abortion, also referred to as ovine enzootic abortion (OEA), results from infection by the C. psittaci pathogen. This organism causes a necrotizing placentitis in sheep and consequent abortion of the lamb. Vaccines prepared from egg-grown Chlamydia psittaci inactivated with Formalin induced immunity in ewes against ovine chlamydial abortion. This vaccine and similar products have been used successfully for decades in sheep to protect against OEA strains of the pathogen. Recently, however, the efficacy of this vaccine has been quite variable, with outbreaks of chlamydial OEA infection occurring in vaccinated flocks. Heterologous challenge experiments have indicated possible strain variation to be the cause.

More recently, protection of sheep against OEA has been demonstrated using a subcellular vaccine containing major outer membrane protein (MOMP) and a subfraction vaccine containing elementary bodies (EBs) from an OEA chlamydial strain (Tan et al., 1990, Infect. Immun., 58:3101-3108). Tan et al. used a modified procedure for isolating chlamydial outer membrane complexes (COMCs) to produce a subcellular vaccine highly enriched in undenatured MOMP. This preparation, given as a single dose containing 20 µg of protein, protected sheep against OEA. A single dose of a vaccine prepared from purified EBs, which contained 160 µg protein, also provided protection against OEA in sheep. Tan et al. identified MOMP

as the major protective component in OEA vaccines and suggested using a recombinant DNA approach to protect against OEA because they believed a 40 kDa MOMP antigen alone was sufficient. While the vaccine disclosed by Tan et al. contained residual amounts of lipopolysaccharide (LPS), this component was not considered important in development of a vaccine against OEA chlamydial infections because serum containing antibodies against LPS did not confer protection in passive transfer experiments, and the complement-fixing antibodies thought to be directed against genus-specific epitopes of LPS did not correlate with protection against ovine abortion strains of C. psittaci.

Attempts to vaccinate against other strains of chlamydial infections in humans and in animals have been even less successful. Several vaccines prepared from attenuated-live or inactivated organisms are available for prevention of C. psittaci infections. However, these vaccines have only resulted in reduction of symptom severity, not in the prevention of disease or elimination of the organism. Vaccination against C. trachomatis in humans using killed, whole elementary bodies has proven to be somewhat protective, however, a delayed-type hypersensitivity has occurred in some cases, exacerbating the disease. Ocular administration of a 57 kDa heat shock protein has been shown to induce a mononuclear cellular inflammatory response in animals. In addition to the heat shock protein, the lipopolysaccharide (LPS) component of Chlamydia has also been shown to contribute to the pathogenesis of the ocular disease caused by the Chlamydia organisms.

While whole virus Chlamydia vaccines have been shown to be somewhat protective, they have also been shown to cause deleterious effects. This has led to the evaluation of individual components for use in a subunit or recombinant vaccine. Polyclonal and monoclonal antibodies to the C. trachomatis major outer membrane protein (MOMP) have been shown to neutralize the ocular infectivity of this organism in a primate model. Oral immunization of monkeys with purified MOMP from C. trachomatis has only resulted in partial protection from subsequent ocular challenge. Due to its protective capacity and the ability of antibodies specific for MOMP to neutralize infectivity, research has focused on the identification of B- and T-cell epitopes on this protein.

It has now been found that the presentation of MOMP from Chlamydia organisms, either alone or in the context of elementary bodies or outer membrane complex, in combination with LPS, is an effective vaccine against chlamydial infections in animals. This combination is more efficient in the induction of a protective response against chlamydial infection than denatured MOMP alone.

Summary of Invention

In one aspect, the present invention provides a vaccine for treatment of chlamydial infections comprising major outer membrane protein and lipopolysaccharide from a Chlamydia organism.

In another aspect, the present invention provides a method for treatment of chlamydial infections comprising administering to an infected animal an effective amount of a vaccine comprising major outer membrane protein and lipopolysaccharide from a Chlamydia organism.

In yet another aspect, the present invention provides a method for immunizing a healthy animal against chlamydial infections comprising administering to a healthy animal a vaccine comprising major outer membrane protein and lipopolysaccharide from a Chlamydia organism.

Detailed Description

The present invention provides a vaccine for treatment and immunization against chlamydial infections in animals. The vaccine is comprised of major outer membrane protein (MOMP) either alone in purified form or in the context of elementary bodies (EBs) or outer membrane complexes (COMCs), with lipopolysaccharide (LPS) from a Chlamydia organism, wherein the Chlamydia organism is preferably C. psittaci or C. trachomatis, more preferably C. psittaci of the Baker strain. The effect of the vaccine may be enhanced by addition of an adjuvant.

The MOMP in the vaccine is provided in purified form or in the context of EBs or COMCs. MOMP can be purified by various methods including but not limited to chromatographically or electrophoretically.

Electrophoretic purification of MOMP (MOMP-E) is accomplished in the following manner. Chlamydia harvest fluid is centrifuged, and the resulting pellet is resuspended in water. Samples are then resolved by gel electrophoresis, preferably sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins in the samples are solubilized, preferably by heating at about 95°C for approximately 5 minutes in buffer, preferably Tris buffer, pH 6.8, containing SDS, 2-mercaptoethanol, glycerol, and bromophenol blue. Electrophoresis is carried out using polyacrylamide gels. The gels are stained, then briefly destained. The 40 kDa band is then excised, placed in a dialysis bag, and the protein is electroeluted out of the gel.

Chromatographic purification of MOMP (MOMP-C) is performed in the following manner. Non-MOMP proteins are extracted from EBs, preferably by

treatment with N-lauroyl-sarcosine in phosphate buffered saline (PBS) containing EDTA for 1 hour at 37°C. Following extraction, the solution is centrifuged. The resulting pellet is washed and resuspended in buffer, preferably sodium phosphate buffer containing MgCl, deoxyribonuclease and ribonuclease A. The suspension is then incubated at about 37°C for approximately 2 hours and spun. The resulting pellet is washed and resuspended in buffer, preferably PBS containing sodium dodecyl sulfate and EDTA. The suspension is incubated again at about 37°C and then spun. The resulting supernatant is dialyzed against buffer, preferably sodium phosphate buffer, containing dithiothreitol and SDS, then loaded onto a hydroxylapatite column previously equilibrated with the same buffer. The column is washed and a linear gradient from about 0.1 to about 0.6 M sodium phosphate, pH 6.4, containing dithiothreitol and SDS is run. The pellet from the SDS extraction is re-extracted. Fractions from the supernatant and the pellet containing MOMP are pooled and then dialyzed against water.

The MOMP preparation in the vaccine can also be provided in the context of EBs or COMCs.

The EB subfraction preparation is isolated from Chlamydia harvest fluid. Chlamydia organisms, preferably Chlamydia psittaci, more preferably Baker strain, is propagated in mammalian cells. The harvest fluid from the cells is concentrated, layered on top of 35% Renografin-76, and centrifuged. The pellet is resuspended, layered on top of a discontinuous gradient of diatrizoate meglunione and diatrizoate sodium and centrifuged. The band at the 44-52% interface which contains the EBs is collected, washed and resuspended in buffer, preferably a phosphate buffer, more preferably 0.01 M phosphate buffer, pH 8.0, containing 0.15 M NaCl (PBS). The EBs are then inactivated, preferably with binary ethylenimine (BEI), β-propiolactone, formalin or glutaraldehyde. If BEI is used in inactivation the solution must be neutralized, preferably by addition of sodium thiosulfate.

To isolate the COMC preparation, the inactivated EBs are centrifuged and the resulting pellet is solubilized, preferably using PBS containing N-lauroyl-sarcosine and EDTA. The solution is then centrifuged, the resulting pellet being washed and resuspended in PBS.

Lipopolysaccharide (LPS) is also added to the vaccine. LPS is isolated via electrophoresis. Samples of the harvest fluid pellet are prepared for electrophoresis as described for the MOMP-E. Electrophoresis is carried out on polyacrylamide gels. It is preferred to use either a Tris/tricine buffer system or a Tris/glycine buffer system with interior resolution. The portion of the gel below the 6 kDa marker is cut off and placed in a basic solution, preferably 0.1 M glycine-NaOH, pH 11.0 and

incubated. The liquid is separated from the gel, the pH is adjusted to neutrality and then dialyzed.

The identity of the antigen is confirmed and the protein concentrations are determined. MOMP-C, MOMP-E, EBs, COMCs and LPS are placed in vials and lyophilized. The amount of LPS is determined by weighing the lyophilized samples. Vials are rehydrated with a pharmaceutically acceptable carrier. Such carriers include normal isotonic saline, standard 5% dextrose in water or water, preferably adjuvanted. Examples of adjuvants include, but are not limited to, Quil A, Alhydrogel, and Quil A and 5% Alhydrogel in tissue culture media. Vials containing LPS are rehydrated first. These solutions are then used to rehydrate the MOMP preparations resulting in a vaccine containing both the MOMP preparation and the LPS preparation.

The vaccine is administered to an animal suffering from a Chlamydial infection. The vaccine is also administered to healthy animals as immunization against infection by a Chlamydia organism. The vaccine can be administered subcutaneously, intramuscularly, intraperitoneally, intravitreally, orally, intranasally or by suppository at doses ranging from 0.01-100 µg/dose of MOMP and LPS each.

As used within the specification, a "MOMP preparation" refers to any vaccine preparation having purified MOMP, including, but not limited to, MOMP-E and MOMP-C, and MOMP in the context of EBs or COMCs. "LPS preparation" refers to a vaccine preparation having purified lipopolysaccharide. "Effective amount" refers to that amount of vaccine which invokes in an animal infected by a Chlamydia organism an immune response sufficient to kill the organism. "Adjuvant" refers to materials which when injected on their own produce a state of nonspecific immunity expressed as a heightened resistance to infection. An example is Quil A in 5% Alhydrogel in tissue culture media.

This invention is further illustrated by the following nonlimiting examples.

Example 1: Subfraction Antigen Preparations

Chlamydia harvest fluid. Chlamydia psittaci, Baker strain was propagated in dog kidney (DK) cells in DMEM with 2% fetal bovine serum. The harvest fluid was inactivated with 1% BEI and the solution neutralized by addition of 0.25% sodium thiosulfate.

Chlamydia elementary bodies (EBs). Non-inactivated Chlamydia harvest fluid was concentrated using a stirred cell concentrator or by centrifugation. The concentrate or pellet was layered on top of 35% Renografin-76 (Squibb Diagnostics, New Brunswick, NJ 08903), and centrifuged at 43,000 g for 1 hour. The pellet was

resuspended and layered on top of a discontinuous gradient of 40, 44 and 52% Renografin-76 (Squibb Diagnostics, New Brunswick, NJ 08903) and centrifuged at 43,000 g for 1 hour. The band at the 44-52% interface containing the EBs was collected, washed and resuspended in 0.01 M phosphate buffer, pH 8.0, containing 0.15 M NaCl (PBS). The EBs were inactivated with 1% BE1 and the solution neutralized by addition of 0.25% sodium thiosulfate.

Chlamydia outer membrane complexes (COMCs). Inactivated EBs were centrifuged at 100,000 g for 1 hour at 10°C, and the resulting pellets solubilized with PBS containing 2% N-lauroyl-sarcosine and 1.5 mM EDTA for 1 hour, at 37°C. The solution was subjected to centrifugation at 100,000 g for 1 hour and the pellet washed once in PBS and then resuspended in PBS.

Example 2: Subunit Antigen Preparation

Chromatographically purified MOMP (MOMP-C). The MOMP was chromatographically purified using a modification of the method described by Caldwell et al. (1981) Infect. Immun., 31:1161-1176. Briefly, EBs prepared as above were treated with 2% N-lauroyl-sarcosine in PBS containing 1.5 mM EDTA for 1 hour at 37°C to extract non-MOMP proteins from the outer membrane complex. The solution was centrifuged at 100,000 g for 1 hour, and the resulting pellet was washed in PBS then resuspended in 3-5 ml of 0.02 M sodium phosphate containing 10 mM MgCl2 and 25 µg each of deoxyribonuclease I and ribonuclease A. This suspension was incubated at 37°C for 2 hours then centrifuged at 100,000 g for 1 hour. The pellet was washed in PBS, then resuspended in 2% sodium dodecyl sulfate (SDS) in PBS with 1.5 mM EDTA and incubated for 1 hour at 37°C. The suspension was centrifuged at 100,000 g for 1 hour. The resulting supernatant was dialyzed again 0.01 M sodium phosphate, pH 6.4, containing 1 mM dithiotreitol and 0.1% SDS (start buffer) and loaded onto a hydroxylapatite column which had been equilibrated in start buffer. The column was washed with start buffer, and then a 150 ml linear gradient of 0.1 to 0.6 M sodium phosphate, pH 6.4, containing 1 mM dithiotreitol and 0.1% SDS was initiated and 1 ml fractions were collected. The pellet from the SDS extraction was re-extracted for 30 minutes each at 37°C with periodic sonication sequentially using each of the following buffers: 1% N-lauroyl-sarcosine in 0.01 M sodium phosphate, pH 7.4; 1% N-lauroyl-sarcosine and 10 mM dithiotreitol in 0.01 M sodium phosphate, pH 7.4; and 1% octylglucoside and 10 mM dithiotreitol in 0.01 M sodium phosphate, pH 7.4 according to the method of Bavoil et al. (1984) Infect. Immuno., 44:479-485. Fractions from the supernatant and the pellet containing MOMP were finally pooled and dialyzed against water.

Electrophoretically purified MOMP (MOMP-E). Chlamydia harvest fluid was centrifuged at 25,000 g and the resulting pellets were resuspended in distilled water. Samples were resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were solubilized by heating at 95øC for 5 minutes in a buffer containing 2% (w/v) SDS, 5% 2-mercaptoethanol (v/v), 3% (w/v) glycerol, 0.002% (w/v) bromophenol blue, and 50 mM Tris (pH 6.8). Electrophoresis was carried out using 10% (w/v) polyacrylamide gels (1.5 mm thick) in the discontinuous buffer system of Laemmli (1970) Nature (London), 227:680-685. The gels were stained with Coomassie Blue G in 50% methanol and 10% acetic acid, and briefly destained in 50% methanol and 10% acetic acid. The 40 kDa band was then excised, placed in a 12-14 kDa molecular weight cut-off dialysis bag and the protein electroeluted out of the gel for 1 hour at 50 V.

Lipopolysaccharide (LPS). Samples of the harvest fluid pellet were prepared for electrophoresis as described for the MOMP-E. Electrophoresis was carried out on 12.5% polyacrylamide gels using the Tris/tricine buffer system of Schagger and von Jagow (1987) Anal. Biochem. 166:368-379, wherein the cathode buffer was 0.1 M Tris, 0.1M Tricine, 0.1% SDS, pH 8.25 and the anode buffer was 0.2M Tris, pH 8.9. The portion of the gel below the 6 kDa marker was cut off and placed in a solution of 0.1 M glycine-NaOH, pH 11.0 and incubated at 37°C for 3 hours. The liquid was then removed from the gel, the pH adjusted to neutrality and then dialyzed against distilled water using 1 kDa molecular weight cut-off dialysis membrane.

Example 3: Quantitation and Evaluation of Protein and LPS

Protein was quantitated using a BCA assay kit (Pierce, P.O. Box 1A, Rockford, Illinois 61105). The amount of LPS was determined by weighing lyophilized samples. Prior to vaccine preparation, antigens were evaluated for purity by staining SDS-polyacrylamide gels with Coomassie Blue G in 50% methanol and 10% acetic acid, and briefly destained in 50% methanol and 10% acetic acid. The identity of the antigens was confirmed by western blot analysis using polyclonal antiserum specific for C. psittaci generated in cats. The procedure involved electrophoretically transferring fractioned antigens from SDS gels onto Immobilon® membranes (Millipore Corporation, Bedford, MA 01730). The membranes were then blocked with 5% instant non-fat dry milk in PBS followed by incubation with the specific antibody for 1 hour at room temperature. Blots were then washed in PBS containing 0.3% Tween (v/v) and incubated with goat anti-cat alkaline phosphatase-labeled antibody (Kirkegaard & Perry Laboratories Inc., 2

Cessna Court, Gaithesburg, Maryland 20879). After extensive washing of the membranes, color was developed using BCIP/NBT substrate (Kirkegaard & Perry Laboratories Inc., 2 Cessna Court, Gaithesburg, Maryland 20879). Stained gels and blots were analyzed by optical image scanning using the Bio Image System (Millipore Corporation, Bedford, MA 01730).

Example 4: Vaccine Preparation

Subfraction antigen preparation for studies I-III were formulated as follows for vaccination of mice. For study I, a specific lot of unprocessed infected tissue culture fluid was used as a positive control since previous studies demonstrated its efficacy. To prepare this vaccine lyophilized fluid was rehydrated with 200 µg/ml Quil A in 5% Alhydrogel in RPMI at a dilution that had been shown to protect mice in past experiments. The total protein present in this dose was 8.1 µg/100 µl. The quantity of EBs in the vaccine was determined by standardizing to the western blot intensity of the MOMP band in the harvest fluid. Based on this, the protein concentration in the EB vaccine was 0.575 µg/100 µl. Three 10-fold serial dilutions of both harvest fluid and EB vaccines were also made.

For study II, EBs and COMCs were formulated as follows. The quantity of COMCs in the vaccine was determined by standardizing to the western blot intensity of the MOMP band in a purified EB preparation. The purified EB preparation was then diluted in PBS to equal the MOMP concentration in COMCs. These were then further diluted in adjuvant (2.5 µg/ml Quil A) in 0.85% NaCl to a total protein concentration of 5 µg/100 µl. The same procedure was followed to produce the COMC vaccine resulting in a protein concentration of 2.8 ug/100 µl and 0.28 µg/100 µl.

To formulate subunit antigens predetermined quantities of each antigen were aliquoted into vials and lyophilized. Vials containing the MOMP-C, MOMP-E and LPS were rehydrated with 200 µg/ml Quil A in 5% Alhydrogel in RPMI medium to yield a concentration of 100 µg/100 µl. Rehydrated LPS was used to rehydrate a few aliquots of the MOMP-E to provide a MOMP-E+LPS vaccine containing 100 µg of each antigen in a 100 µl total volume. Three 10-fold dilutions of the above 4 vaccines were made in 200 µg/ml Quil A in 5% Alhydrogel diluted in RPMI. All vaccines were stored at 4°C between the time of preparation and administration (1 day for the first dose and 14 days for the second dose).

Example 5: Vaccination and Challenge

Female Swiss White CF-1 mice weighing 12-14 grams (Charles River) received 2 vaccinations of 100 µl subcutaneously, two weeks apart. There were 8 mice in each group with the exception of the 10 µg group for the MOMP-C, MOMP-E, LPS, MOMP-E+LPS groups, the 0.058 µg group for the EB and the 0.81 æg group for the harvest fluid in which there were 10 mice per group. The 2 additional mice in these groups were sacrificed and bled on the day the remainder of the mice were challenged. Vaccinates as well as 10 controls were challenged with an intraperitoneal inoculation of C. psittaci, Cello strain (Cello (1967) Am. J. Ophthalmol., 63:1270-1273) 2 weeks after the second immunization.

For each study, 3 additional groups of 10 mice were challenged with 10-fold serial dilutions of the challenge material to confirm the LD_{50}. At the dilution of the Chlamydia organisms used to challenge vaccinates and at a 1:10 dilution of this challenge material all the control mice (10 per group) died.

All subunit preparations, except LPS, protected 100% of the mice when administered at 100 µg/dose. A dose-related titration of the protective effect was also observed. Comparison of the various MOMP-containing preparations administered at the 10 µg dose indicated that both MOMP-C alone and MOMP-E plus LPS still protected 100% of the mice, while MOMP-E could only induce a 60% protective level. Comparable levels of protection were also observed with the 1.0 µg and 0.1 µg doses of the MOMP-C alone and MOMP-E plus LPS preparations. These levels were significantly greater than that induced by MOMP-E alone. See data provided in Table 1.

TABLE 1

Protection of Mice with Chlamydia Subunit Preparations

Subunit Preparation Antigen/Adjuvant	Total Protein (µg)	ProtectionLevel (%) # survivors/total
MOMP-C 2% ALOH, 25 µg Quil A	100	8/8(100)
	10	8/8 (100)
	1.0	6/8 (75)
	0.1	0/8 (0)
MOMP-E 2% ALOH, 25 µg Quil A	100	8/8 (100)
	10	5/8 (62.5)
	1.0	3/8 (37/5)
	0.1	0/8 (0)
LPS 2% ALOH, 25 µg Quil A	100	3/8 (37/5)
	10	3/8 (37/5)
	1.0	0/8 (0)
	0.1	2/8 (25)
MOMP-E + LPS 2% ALOH, 25 µg Quil A	100	8/8 (100)
	10	8/8 (100)
	1.0	5/8 (63.5)
	0.1	1/8 (12.5)

Example 6: Evaluation of Immune Response by ELISA

EBs were fixed with methanol to 96-well round-bottom Immulonþ 2 plates (Dynatech Laboratories, 14340 Sullyfield Circle, Chantilly, Virginia) at 1 µg/well. Plates were washed with distilled water, then blocked with 3% horse serum (Hy-Clone Laboratories, Inc., 1725 South HyClone Road, Logan, Utah 84321) in PBS for 1 hour at room temperature. Mouse sera was diluted in PBS containing 0.3% Tween (v/v) and applied to plates. After a 1 hour incubation at room temperature, plates were washed with PBS containing 0.3% Tween and further incubated with peroxidase-labeled antiserum to mouse IgG (Kirkegaard & Perry Laboratories Inc., 2 Cessna Court, Gaithesburg, Maryland 20879). Plates were then washed with PBS containg 0.3% Tween and developed with ABTS substrate (Kirkegaard & Perry Laboratories Inc., 2 Cessna Court, Gaithesburg, Maryland 20879). After 30 minutes, the O.D. 405-490 was measured. Titers are expressed as the dilution of

mouse serum giving an O.D. reading of 0.200. Background values were consistently between 0.002-0.004 O.D. units.

Serum titers were determined for two randomly chosen mice from each dose group prior to being challenged. A correlation between the ability of the vaccines to protect mice from infection and the EB-specific serum response was observed. Western blot analysis from mice vaccinated with MOMP-C alone and MOMP-E plus LPS indicated that the response was almost exclusively directed to the MOMP.

What is claimed is:

1. A vaccine for treatment of chlamydial infections comprising a MOMP preparation and a LPS preparation from a Chlamydia organism.

2. The vaccine of claim 1 wherein the MOMP preparation is selected from a group consisting of MOMP-E, MOMP-C, and MOMP in the context of EBs or COMCs.

3. The vaccine of claim 2 wherein the MOMP preparation comprises MOMP in the context of EBs.

4. The vaccine of claim 2 wherein the MOMP preparation comprises MOMP-E.

5. The vaccine of claim 1 wherein the Chlamydia organism comprises Chlamydia psittaci.

6. The vaccine of claim 5 wherein the Chlamydia psittaci comprises a Baker strain.

7. The vaccine of claim 1 further comprising an adjuvant.

8. A vaccine for treatment of chlamydial infections comprising MOMP in the context of EBs, LPS and an adjuvant.

9. A method for treatment of chlamydial infections comprising administering to an animal infected with a Chlamydia organism an effective amount of a vaccine comprising a MOMP preparation and a LPS preparation from a Chlamydia organism.

10. The method of claim 9 wherein the MOMP preparation in the vaccine is selected from a group consisting of MOMP-E, MOMP-C, and MOMP in the context of EBs or COMCs.

11. The method of claim 10 wherein the MOMP preparation in the vaccine comprises MOMP in the context of EBs.

12. The method of claim 10 wherein the MOMP preparation in the vaccine comprises MOMP-E.

13. The method of claim 9 wherein the Chlamydia organism comprises Chlamydia psittaci.

14. The method of claim 13 wherein the Chlamydia psittaci comprises a Baker strain.

15. The method of claim 9 wherein the vaccine further comprises an adjuvant.

16. A method for treatment of chlamydial infections comprising administering to an animal infected with a Chlamydia organism an effective amount of a vaccine comprising MOMP in the context of EBs, LPS and an adjuvant.

17. A method for immunizing an animal against chlamydial infections comprising administering to a healthy animal a vaccine comprising a MOMP preparation and a LPS preparation from a Chlamydia organism.

18. The method of claim 17 wherein the MOMP preparation in the vaccine is selected from a group consisting of MOMP-E, MOMP-C and MOMP in the context of EBs or COMCs.

19. The method of claim 18 wherein the MOMP preparation in the vaccine comprises MOMP in the context of EBs.

20. The method of claim 18 wherein the MOMP preparation in the vaccine comprises MOMP-E.

21. The method of claim 17 wherein the Chlamydia organism comprises Chlamydia psittaci.

22. The method of claim 21 wherein the Chlamydia psittaci comprises a Baker strain.

23. The method of claim 17 wherein the vaccine further comprises an adjuvant.

24. A method for immunizing an animal against chlamydial infections comprising administering to a healthy animal a vaccine comprising MOMP in the context of EBs, LPS and an adjuvant.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 39/118
US CL :424/263.1, 282.1, 831
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/263.1, 282.1, 831

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, Embase, Derwent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	Infection and Immunity, Volume 58, Number 9, Issued September 1990, Tan et al, "Protection of Sheep Against *Chlamydia psittachi* infection with a subcellular vaccine containing the major outer membrane protein", pages 3101-3108, see pages 3101-3104.	24 ------ 1-3, 5-11, 13-19, 21-24
Y	Infection and Immunity, Volume 31, Number 3, Issued March 1981, Caldwell et al, "Purification and Partial Characterization of the Major Outer Membrane Protein of *Chlamydia trachomatis*", pages 1161-1176, see page 1163.	1-3, 5-11, 13-19, 21-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
15 JANUARY 1995

Date of mailing of the international search report
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Infection and Immunity, Volume 54, Number 2, Issued November 1986, Brade et al, "Chemical, Biological, and Immunochemical Properties of the *Chlamydia psittaci* Lipopolysaccharide", pages 568-574, see page 569.	1-3, 5-11, 13-19, 21-24
Y	The Journal of Immunology, Volume 114, Number 2, Part 2, Issued February 1975, Skidmore et al, "Immunologic Properties of Bacterial Lipopolysaccharide (LPS): Correlation Between the Mitogenic, Adjuvant, and Immunogenic Activities", pages 770-775, see page 770.	1-3, 5-11, 13-19, 21-24
Y	Buck et al, "AMERICAN *TYPE CULTURE* COLLECTION CATALOGUE OF ANIMAL VIRUSES AND ANTISERA, CHLAMYDIAE AND RICKETTSIAE", 6th edition published 1990 by American Type Culture Collection (Rockville, MD), pages 171-174.	6, 14, and 22
Y	US, A, 4,271,146 (SEAWELL) 02 June 1981, see columns 1 and 3.	1-3, 5, 7-11, 13, 15, 16
A	British Medical Bulletin, Volume 39, Number 2, Issued April 1983, Woodland et al, "Animal Models of Chlamydial Infection", pages 175-180, see pages 176, 177 and 179.	1-24
A	Infection and Immunity, Volume 58, Number 5, Issued May 1990, Baghian et al, "Antibody Response to Epitopes of Chlamydial Major Outer Membrane Proteins on Infectious Elementary Bodies and of the Reduced Polyacrylamide Gel Electrophoresis-Separated Form", pages 1379-1383, see page 1382.	1-24
A	Davis et al, "MICROBIOLOGY: INCLUDING IMMUNOLOGY AND MOLECULAR GENETICS", 3rd edition, published 1980 by Harper and Row, Publishers, Inc. (Hagerstown, MD), see pages 776-784.	1-24